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Nitrate concentration-related safety of drinking water from various sources intended for consumption by neonates and infants

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Abstract: The aim of the paper is to compare nitrate concentrations in samples of supply water as well as water from deep and dug wells located in the eastern region of Poland. Additionally, samples of bottled water (spring and natural mineral), certified by the Institute of Mother and Child and the Children's Memorial Health Institute, were subjected to analyses. On the basis of the obtained results, health risks related to the occurrence of methemoglobinemia in neonates and infants were evaluated. The risk analysis was performed according to the procedure recommended by the United States Environmental Protection Agency. Nitrate concentrations in the examined samples ranged from: 0.153–161.1 mg/l. The lowest concentration of nitrates was determined in the samples of bottled water, the highest being detected in the water from dug wells. It was found that nitrate concentration in samples of bottled and supply water did not pose any risk to the health of neonates and infants. The highest health risk related to methemoglobinemia occurs for neonates consuming water originating from dug wells. The risk decreases along with the age of an infant.

Introduction

Water is a substance that is essential for life. It is a carrier of nutrients and various compounds formed in metabolic processes. Water content in the human body varies and changes with age. Neonates and infants' bodies contain the highest amounts of water (84–64%). For comparison, bodies of adults aged 50 or over contain much less water – in men: 47–67% and in women: 39–57% (EFSA 2010a). The water demand of infants, in relation to the body weight, is four times as high as in adults. In the nutrition of infants and children, drinking water is recommended to be of good quality. For infants and children under the age of three, the best water for consumption is spring water or natural mineral water (Woś et al. 2010). It is characterized by the natural content of components solved in it. The natural content of components in groundwater in Poland (assumed as the hydrogeochemical background) is specified in the Regulation of the Minister of Environment of 23 July 2008 on the criteria and methods for evaluating the condition of groundwater (Dz. U. 2008, No. 143, item 896).

The quality of water intended for consumption should satisfy the requirements provided in the Regulation of the Minister of Health of 13 November 2015 on the quality of water intended for human consumption (Dz. U. 2015, item 1989). This regulation imposes on the State Sanitary Inspection the obligation to carry out evaluations of the quality of drinking water from the water supply systems and from individual intakes supplying water for at least 50 people or providing at least 10 m^3 water on average per day.

Water is safe for consumption by humans if it is free from pathogenic microorganisms and parasites, as well as from other substances such as uranium (Garboś and Święcicka 2015) and nitrates (Kiryluk 2011, US EPA 1986, 1991, Ward et al. 2005) in amounts posing a potential threat for health. Nitrates in the human body are enzymatically reduced to nitrites with bacterial mediation. Nitrites oxidise the ferrous iron (Fe^{2+}) in haemoglobin (Hb) to the ferric form (Fe^{3+}) resulting in the formation of methaemoglobin (MetHb). The trivalent iron ion in the composition of methaemoglobin has no oxygen-binding ability. Methemoglobinemia symptoms include: headache, cyanosis, stupor, cerebral anoxia, tachycardia, syncope, convulsions, lethargy, coma and death (Mensinga et al. 2003, Gupta et al. 2008). The problem of the toxic effect of nitrates mostly concerns infants in their first months of life (Mensinga et al. 2003, Bryan and van Grinsven 2013) and it results directly from several reasons: their higher gastric pH, a higher proportion of foetal haemoglobin (foetal Hb) (above 50%) in total haemoglobin, which shows a higher resistance to oxidizing, and the fact that infants consume more water in relation to their body weight than adults. Additionally, infants under 3 months of age have a poorly-developed enzymatic system for reducing methaemoglobin to haemoglobin (Mensinga et al. 2003).

In the nutrition of infants and small children, it is recommended to use good quality drinking water. Water available for sale includes bottled waters certified by the Institute of Mother and Child, the Children's Memorial Health Institute and the State Institute of Hygiene. Labels on the packages of water intended for consumption by small children do not contain information specifying their nitrate concentration.

The main source of drinking water for inhabitants of Poland is water from water supply intakes subject to inspection by the State Sanitary Inspection (Dz. U. 2015, item 1989). Data provided by the Central Statistical Office (GUS 2015a) shows that 91.6% of the total population used the water supply networks in 2014. More than 96% of urban population and over 84% of people living in rural areas had access to supply water networks. The rural population also uses private wells providing drinking water of unknown physico-chemical and microbiological parameters. According to the literature (Bilek and Rybakowa 2014, Bilek et al. 2015, Jaszczyński et al. 2006, Raczuk 2010), in Poland, water from well intakes, particularly from dug wells, has high concentrations of nitrates which often exceed the standards. It must be emphasized that in Poland, the natural concentration of nitrates in drinking water is assumed at the level of 5 mg/l (Dz. U. 2008, No. 143, item 896).

In view of the high percentage of water in the infant body and particularly high sensitivity and rapid response of the body in this age group to the presence of nitrates, many studies have concerned the evaluation of health risks related to the supply of nitrates with drinking water (Bozek at al. 2013, Pawełczyk 2012, Su et al. 2013).

The aim of the research was to compare the concentration of nitrates in samples of drinking water from various sources in selected provinces of eastern Poland, as well as to assess health risks in neonates and infants related to the exposure to nitrates.

Materials and methods

Samples of drinking water were taken in the areas of the following provinces: Warmia and Mazury (n=21), Podlaskie (n=35), Lublin (n=45), Subcarpathian (n=23) and the eastern part of Mazovia (n=63) (Fig. 1). The percentage of the population using the water supply network in the examined area ranges from 80% (Podkarpacie) to 94% in Warmia and Mazury (GUS 2015a). The total amount of 1.5 l water samples were collected in 2013–2014 at random from each of 104 water supply intakes, from 58 dug wells with depths ranging from 5 to 20 m and from 25 deep wells with depths ranging from 26 to 56 m. Samples from the water supply system were collected both from rural and urban networks, while samples of well-water were taken mainly in rural areas. The analysis also included nine samples of commercially-available bottled water, certified by the Institute of Mother and Child and the Children's Memorial Health Institute. These included the following spring waters: Baby Zdrój, Mama i Ja, Nestle Pure, Jurajska Junior, Primavera, Sugaro Junior, Żywiec Zdrój (Zdrojek) and the natural mineral waters: Polaris Plus and Polaris Kids.

Nitrate levels in water samples were spectrophotometrically determined with phenol disulphonic acid within concentrations ranging from 0.02 to 2.00 mg $N\text{-}NO_3^-$/ml. The absorbance of radiation was measured with a Shimadzu UV-1800 spectrophotometer, at the 410 nm wavelength. The analysis of each sample was performed in three replications. The accuracy of the determination of nitrates in water samples was checked with the method of standard addition.

The results of the study were statistically analysed. They were also used to evaluate the risk to infant health related to exposure to nitrates in drinking water. The evaluation of the health risk was performed according to the procedure

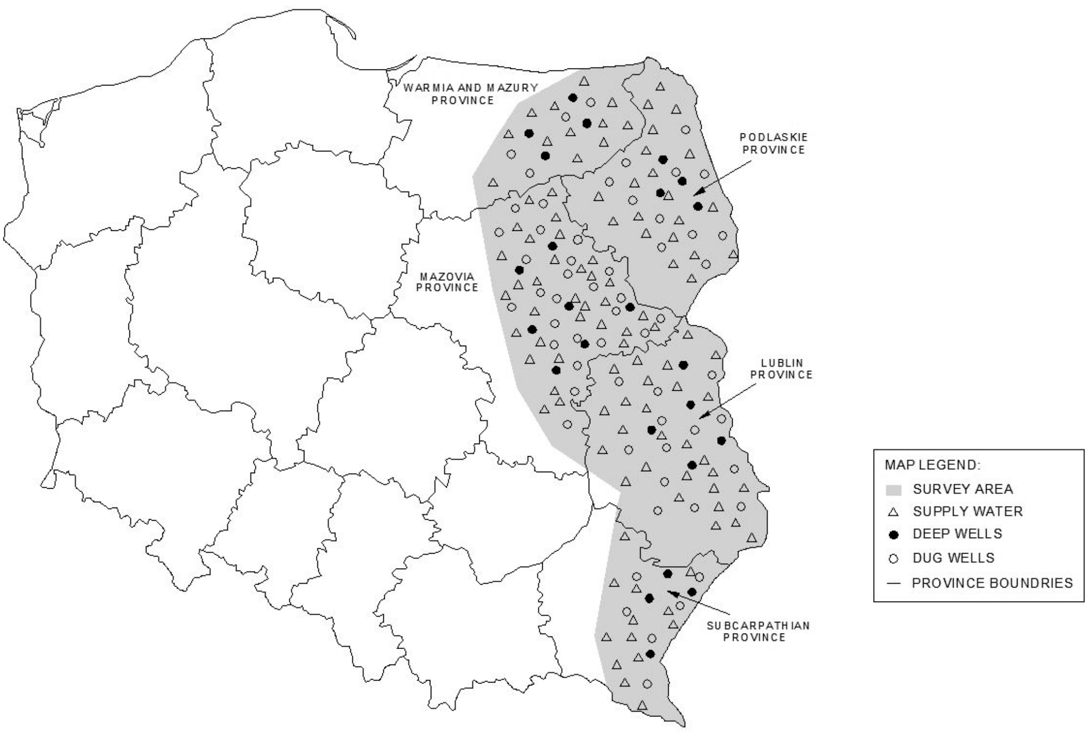

Fig. 1. Study area and sampling sites

recommended by the United States Environmental Protection Agency (US EPA 1989).

The results were statistically analysed with Statistica 2012 software. Since a Shapiro-Wilk test demonstrated that the variables did not have a normal distribution ($p < 0.01$), a non-parametric Kruskal-Wallis was applied to compare the values of the studied parameters. In further statistical calculations the Dunn's multiple range test was used. The value of the correlation coefficient between the nitrate level and the depth of the well was calculated using the Spearman (R_s) range test.

A risk scenario was developed taking into account the age group which is the most susceptible to excessive nitrates in water, represented by neonates and infants below the age of one. It was assumed that they were bottle-fed children. The number of live births in 2014 ranged from 13,958 in Warmia and Mazury to 57,139 in Mazovia (GUS 2015b).

The research methodology of health risk assessment applied in the study was based on the procedure recommended by the United States Environmental Protection Agency (US EPA 1989). Toxicological data concerning nitrates were derived from the Integrated Risk Information System (IRIS) database (US EPA1991), which is recommended as the preferred source of toxicological information. Since nitrates are classified as group D chemical substances according to US EPA (1986; 1991), for which there is insufficient data confirming their potential carcinogenicity, the health risk evaluation process was based on measuring exposure to toxic substances, although not carcinogenic ones.

In order to assess health risks related to nitrates present in drinking water, the following formula reflecting the intake of threshold substances with drinking water (Pawełczyk 2012, US EPA1989) was used:

$$ADD = C \times FI \times (IR \times EF \times ED) / (BW \times AT)$$

where:
ADD – average daily dose of nitrates taken in with drinking water (mg/kg/day),
C – concentration of NO_3^- ions in drinking water in the exposure period (mg/l),
FI – abstract number from 0 to 1, describing what part of the actual intake originates from the polluted source; the analysis assumed FI = 1,
IR – the intake rate of drinking water consumed per day [mg/], assumed, according to US EPA (2008) recommendations, 95th percentile for acute exposure, i.e. the maximum water intake by neonates and infants (Table 1),
EF – the exposure frequency – 7 days/week for all age groups of infants and neonates,
ED – the exposure duration – for all age groups of infants and neonates ED = 1 week,
BW – the average body weight [kg], assumed for each age group according to the US EPA (2008) recommendations (Table 1),
AT – the average time (in days), for which the average is calculated – the same value of AT = 7 days was assumed for analysing the acute exposure for all age groups of infants and neonates.

The level of risk related to the current acute exposure was evaluated using the hazard quotient (HQ), calculated according to the formula:

$$HQ = ADD/RfD$$

where:
ADD – average daily dose (mg/kg/d),
RfD – oral reference dose (mg/kg/d). According to US EPA (1991), a reference dose for nitrates in drinking water is 1.6 mg $N\text{-}NO_3^-$/kg/d i.e. 7.09 mg NO_3^-/kg/d.

Results

Nitrate concentrations in the examined samples ranged from 0.153–161.1 mg/l (Table 2). In all examined samples of bottled water recommended for infants and small children, the level of nitrates did not exceed 5 mg/l. It also did not exceed the value in 86.5% of supply water samples, 72% of deep well water samples and 3% of samples taken from dug wells. In about

Table 1. Exposition factors for individual age group (neonates and infants) (US EPA 2008)

Age group	Month	Body weight (BW) [kg]	Intake rate (IR) of drinking water [l/d]
Neonates	<1	4.8	0.839
Infants	1–3	5.9	0.896
	3–6	7.4	1.056
	6–12	9.2	1.055

Table 2. Concentration of nitrate in water samples

Samples water	N	[mg NO_3^-/l]						
		Min	Max	Mean	SD	Median	Lower Quartile	Upper Quartile
Bottled water	9	0.226	1.413	0.659	0.422	0.443	0.985	0.340
Supply water	104	0.153	24.44	2.714	4.107	1.121	0.682	3.356
Deep wells	25	0.512	47.12	9.509	14.27	3.720	1.820	11.82
Dug wells	58	2.451	161.1	43.52	33.11	32.00	19.46	62.52

35% of water samples from dug wells, the concentration of nitrates exceeded the normative value of 50 mg/l (Fig. 2). In water samples taken from various sites statistically significant differences ($H_{3,196}$ = 117,99; p = 0.0000) of nitrate concentrations were noted. The concentration of nitrate in bottled water samples was significantly lower than nitrate concentration in water samples from deep (p = 0.0052) and dug (p = 0.0000) wells. No significant differences in nitrate concentrations were observed in bottled and supply water samples. The differences were significant in the case of supply water samples and the samples from dug (p = 0.0000) as well as deep (p = 0.0249) wells. Nitrate concentration in the samples of water from deep wells was significantly lower than the one in dug wells water (p = 0.0001).

Statistical analysis did not show differences in nitrate concentrations in supply, dug and deep water samples taken from various provinces. However, within individual provinces, significant differences in nitrate concentrations were noted in water samples collected at different sites: Mazovia ($H_{2,63}$ = 37,75; p = 0.0000), Podlasie ($H_{2,35}$ = 21.08, p = 0.0000), Warmia and Mazury ($H_{2,21}$ = 11,92; p = 0.0026), Lublin ($H_{2,45}$ = 28.03; p = 0.0000), Subcarpathian ($H_{2,23}$ = 10.08, p = 0.0065). In water from each province, significant differences ($p < 0.005$) in nitrate concentrations were observed in supply water samples and the samples taken from dug wells. What is more, in the samples from Mazovia region, differences in nitrate concentrations were detected in dug and deep wells water (p = 0.0132). It should also be noted that nitrate concentration in water samples from dug wells was significantly negatively correlated (R_s = -0.673, $p < 0.05$) with the depth of the well. Lower concentrations of nitrates were recorded in samples of water originating from deeper wells. Such a relation was not found for deep wells.

The hazard quotient (HQ) calculated on the basis of the average daily dose (ADD) of nitrates with drinking water for various age groups of infants and the oral reference dose (RfD) for nitrates are presented in Table 3. The calculated value of HQ < 1 means that the estimated exposure to nitrates in drinking water is lower than RfD and that the risk is acceptable. The hazard quotient (HQ) for each age group of infants and neonates drinking spring water, natural mineral water and supply water was below 1. The consumption of water from 3% of deep wells posed a danger to neonates and infants below their third month of life. For other groups of infants, the intake of nitrates with water from deep wells did not pose a threat to life. For dug wells, the intake of water from 42% of the examined wells posed a threat to neonates, while water from 36% of intakes posed a threat to infants under three months of age. The percentage of well-water samples posing a threat to infant health was the lowest for the group above six months of age (Fig. 3). The highest health risk caused by nitrates according to the assumed threat scenario was borne by new-born babies, for which the maximum hazard quotient (HQ) was 3.972 (Table 3).

Discussion

Drinking water quality is an important element of human health in view of its permanent place in everyday nutrition. Drinking water can be a source of macro- and microelements for the human body (Ćurković et al. 2016, Rosborg 2015). It can also contain substances that are hazardous to health and which in concentrations exceeding norms can pose a threat to the health of consumers (Bukowski et. al. 2001, Ćurković et al. 2016, Garboś and Święcicka 2015, Ward et al. 2005). For this reason, it is subject to evaluation by the State Sanitary Inspection. According to the Regulation of the Minister of Health (Dz. U. 2015, item 1989), water from the water supply system should be examined at least four times a year. Applicable standards determining the parameters of acceptable levels of substances that are hazardous to health take into consideration the recommendations of the World Health Organisation and are consistent with the EU Council Directive 98/83/EC (Gromiec 2004). The analysed samples of bottled waters satisfied the applicable norms of 50 mg NO_3^-/l for spring water and 10 mg NO_3^-/l for natural mineral water (Dz. U. 2011, No. 85, item 466). Nitrate concentrations in the examined bottled waters did not exceed the hydrogeochemical background level.

All samples of supply water in terms of nitrate concentration met the recommended norm -50 mg/l (Dz. U.

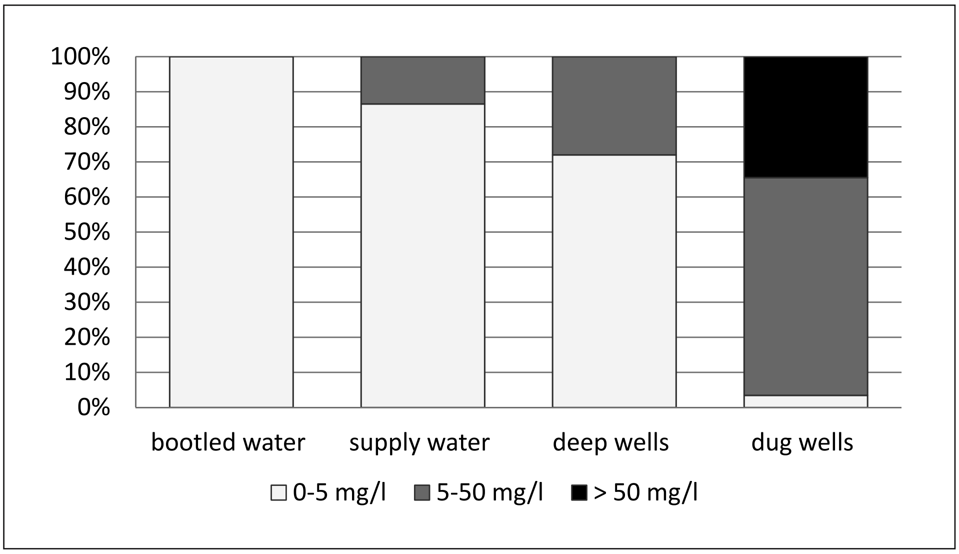

Fig. 2. Percentage share of water samples of different concentration of nitrates

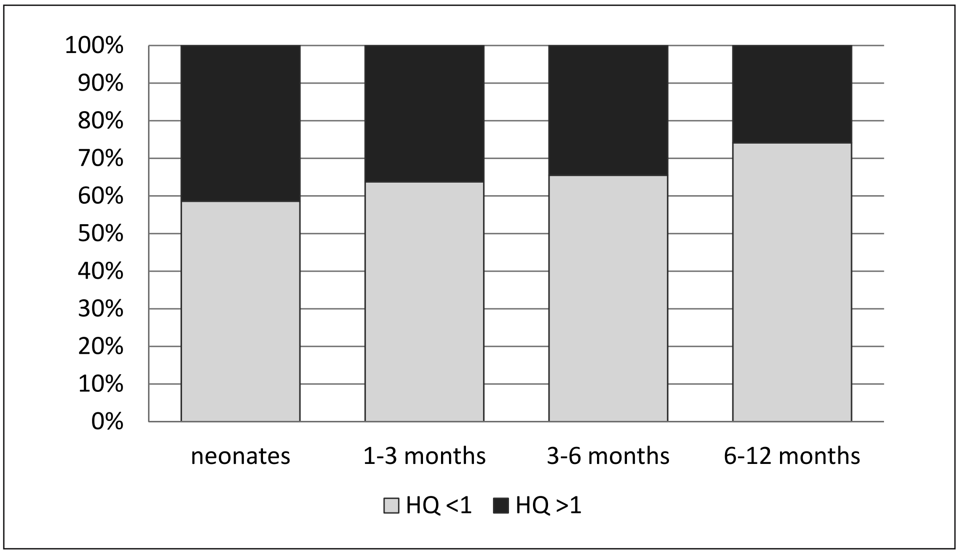

Fig. 3. Percentage share of water samples of different hazard quotients (HQ) for nitrates in dug wells water for the age group of infants and neonates

Table 3. Average daily dose (ADD) and hazard quotients (HQ) for nitrates in drinking water

Samples water	Age [month]	ADD [mg NO_3^-/l]	HQ
		Range	Range
Bottled water	<1	0.040–0.247	0.006–0.035
	1–3	0.034–0.215	0.005–0.030
	3–6	0.032–0.202	0.005–0.028
	6–12	0.026–0.162	0.004–0.023
Supply water	<1	0.027–4.272	0.004–0.603
	1–3	0.023–3.712	0.003–0.523
	3–6	0.022–3.488	0.003–0.492
	6–12	0.012–2.803	0.002–0.395
Deep wells	<1	0.090–8.236	0.013–1.162
	1–3	0.078–7.156	0.011–1.009
	3–6	0.074–7.724	0.010–0.948
	6–12	0.059–5.403	0.008–0.762
Dug wells	<1	0.428–28.16	0.060–3.972
	1–3	0.372–24.47	0.049–3.243
	3–6	0.350–22.99	0.049–3.243
	6–12	0.281–18.47	0.040–2.606

2015, item 1989). In supply water samples taken in urban areas, the average concentration of nitrates (2.62 mg/l) was comparable to the average concentration of nitrates (2.77 mg/l) in water samples taken from rural water supply systems. The highest concentration of nitrates ranging from 18.80 to 24.40 mg/l was found in three samples of water originating from two rural water supply systems (Domanice and Sarnaki) and from a small town (Kałuszyn). According to the literature data (Szczerbiński et al. 2006), the health risks related to the presence of nitrates in drinking water originating from water supply systems are higher for inhabitants of rural areas than for town dwellers. Radzka et al. (2015) found nitrate concentrations above the norms recorded in 20% of supply water samples in the Siedlce District. The results of the nitrate level monitoring of groundwater in areas particularly exposed to contamination with nitrogen compounds from agricultural sources demonstrated that in 2014, 10.6% of the examined samples exceeded 50 mg/l (GUS 2015c).

Nitrate concentrations in supply water samples were significantly lower than nitrate levels in samples of water taken from dug and deep wells. Water samples from 28% of deep wells demonstrated nitrate concentrations above the hydrogeochemical background, which indicates an inflow of anthropogenic pollution. In 4 out of 5 provinces water samples were taken from, no significant differences in nitrate concentrations in water samples from dug and deep wells were noted.

The highest concentration of nitrates (above 100 mg/l) was recorded in water samples from two 10–12 m deep wells. The inflow of nitrates from area sources (farming fields fertilized with organic and mineral fertilizers) and spot sources (leaking septic tanks, farm buildings and manure piles) especially affects shallow dug wells (Jaszczyński et al. 2006, Kiryluk 2011, Raczuk 2010, Sapek 2004).

Well-water is usually used by individual consumers. Water from such intakes is not subject to statutory examination by the State Sanitary Inspection (Dz. U. 2015, item 1989). Assessments of well-water quality conducted in agricultural areas by e.g. Raczuk (2010) and Bilek et al. (2015) demonstrated nitrate concentrations exceeding acceptable levels in more than 70% of samples. In the results presented in this study, excessive nitrate level was recorded in 35% of the examined samples of water taken from dug wells. This means that nitrate concentration in more than 40% of the examined water samples poses a health risk to neonates. This risk is significantly reduced to about 26% in the group of infants aged 6–12 months. Su et al. (2013), in the research on agricultural areas of the north-eastern China, demonstrated that the consumption of nitrates in drinking water posed a higher risk to infants and children than to adults. Ingested nitrates converted to nitrite by microflora increased infant mortality, spontaneous abortions, birth defects, recurrent diarrhoea, recurrent stomatitis, histopathological changes in cardiac muscles, alveoli of lungs and adrenal glands, deterioration of the immune system of the body and increased free oxide radicals that predispose cells to irreversible damage and effects such as cancer (Gupta et al. 2008). According to Brender et al. (2013) whose studies were carried out in the area of Texas and Iowa, children whose mothers consumed nitrates in drinking water in pregnancy have a higher risk of cleft palate, spina bifida and other birth defects.

Drinking water with an unknown nitrate level should not be used for preparing food for neonates and bottle-fed infants and should not be drunk by infants under the age of one. Additionally, such water should not be consumed by pregnant women. Apart from drinking water, vegetables might be a negative source of nitrates in the diet of neonates aged over 3 months and of pregnant women (EFSA, 2010b, Hord et al. 2009).

Conclusions

1. The conducted analysis of drinking water originating from various sources in the aspect of providing it to neonates and infants demonstrated that bottled water certified by the Institute of the Mother and Child and the Children Health Institution is safe for consumption by neonates and infants and pregnant women as regards its nitrate levels.
2. The examined supply water samples did not pose a threat to neonates and infants in terms of nitrate concentration.
3. The assessment of health risk according to the assumed scenario demonstrated that neonates and infants consuming water from dug wells might be most exposed to the risk of methemoglobinemia. This health risk lowers with the age of the infant.
4. Consumption of drinking water from private intakes, mainly from deep wells, can pose a health risk to neonates and infants. In view of a lack of statutory inspection of water from privately owned wells, in the absence of appropriate analyses of water samples, it is advisable to provide infants with bottled water recommended for consumption by the above-mentioned institutions.

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Stężenie azotanów w wodzie z różnych źródeł przeznaczonej do spożycia dla noworodków i niemowląt w aspekcie bezpieczeństwa zdrowotnego

Streszczenie: Porównano stężenie azotanów w próbkach wody wodociągowej, ze studni głębinowych i kopanych zlokalizowanych na obszarze wschodniej Polski oraz w wody butelkowanej (źródlana i naturalna mineralna), posiadającej atest Instytutu Matki i Dziecka i Centrum Zdrowia Dziecka. Na podstawie otrzymanych wyników dokonano oceny ryzyka zdrowotnego, związanego z występowaniem methemoglobinemii u noworodków i niemowląt. Ocenę ryzyka wykonano zgodnie z procedurą rekomendowaną przez Amerykańska Agencję Ochrony Środowiska. Stężenie azotanów w badanych próbkach wody zmieniało się w zakresie: 0,153–161,1 mg/l. Najmniejsze stężenie azotanów oznaczono w próbkach wody butelkowanej, największe odnotowano w próbkach wody studziennej. Ustalono, że stężenie azotanów w próbkach wody butelkowanej i wodociągowej nie stanowi zagrożenia dla zdrowia noworodków i niemowląt. Największe ryzyko zdrowotne związane z narażeniem noworodków na methemoglobinemię może występować w przypadku spożycia wody ze studni kopanych. Zmniejsza się ono wraz z wiekiem niemowlęcia.